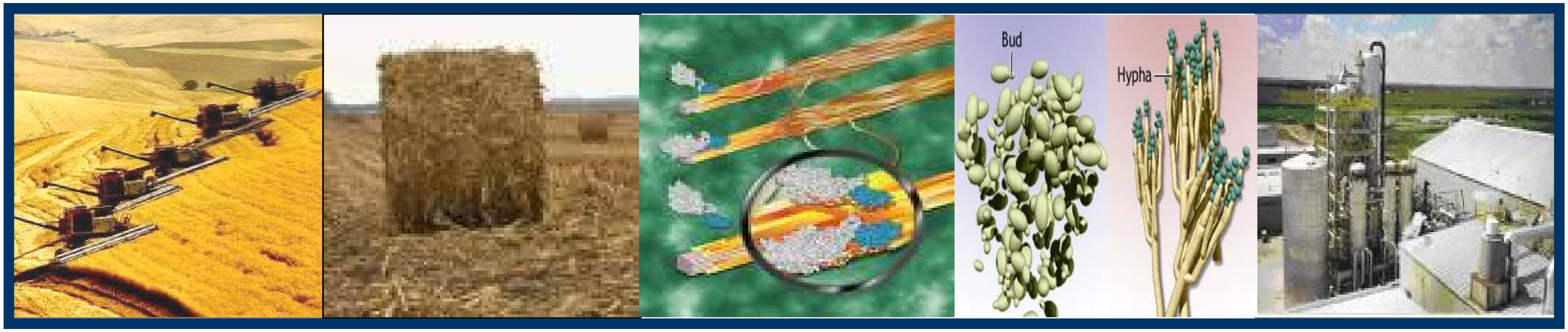

Fully Integrated Lignocellulosic Biorefinery with *Onsite* Production of Enzymes and Yeast

Manoj Kumar, PhD
Director Science & Technology
Global New Business Development
White Biotechnology
Royal DSM

INTERNATIONAL FEW FUEL ETHANOL WORKSHOP® & EXPO a BBI International event

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DSM: Company Profile 2009
Life Sciences and Materials Sciences Company

- **Global top 30 chemical industry**
- Net sales : $13,692 million
- Net earnings: $895 million
- **23,500 employees**
  - R&D: approx. 2,130
  - in the Netherlands: approx. 7,200
  - in the US: approx. 3,000
- >200 locations on 5 continents
- **Among Top Three listed in Dow Jones Sustainability Index** in 2004, 2005, 2006, 2007, 2008, 2009
- **Strong technological toolbox**: **Integrated use of biotechnology, biocatalysis, organic chemistry, chemical and polymer technology, materials sciences**

World Business Council for Sustainable Development

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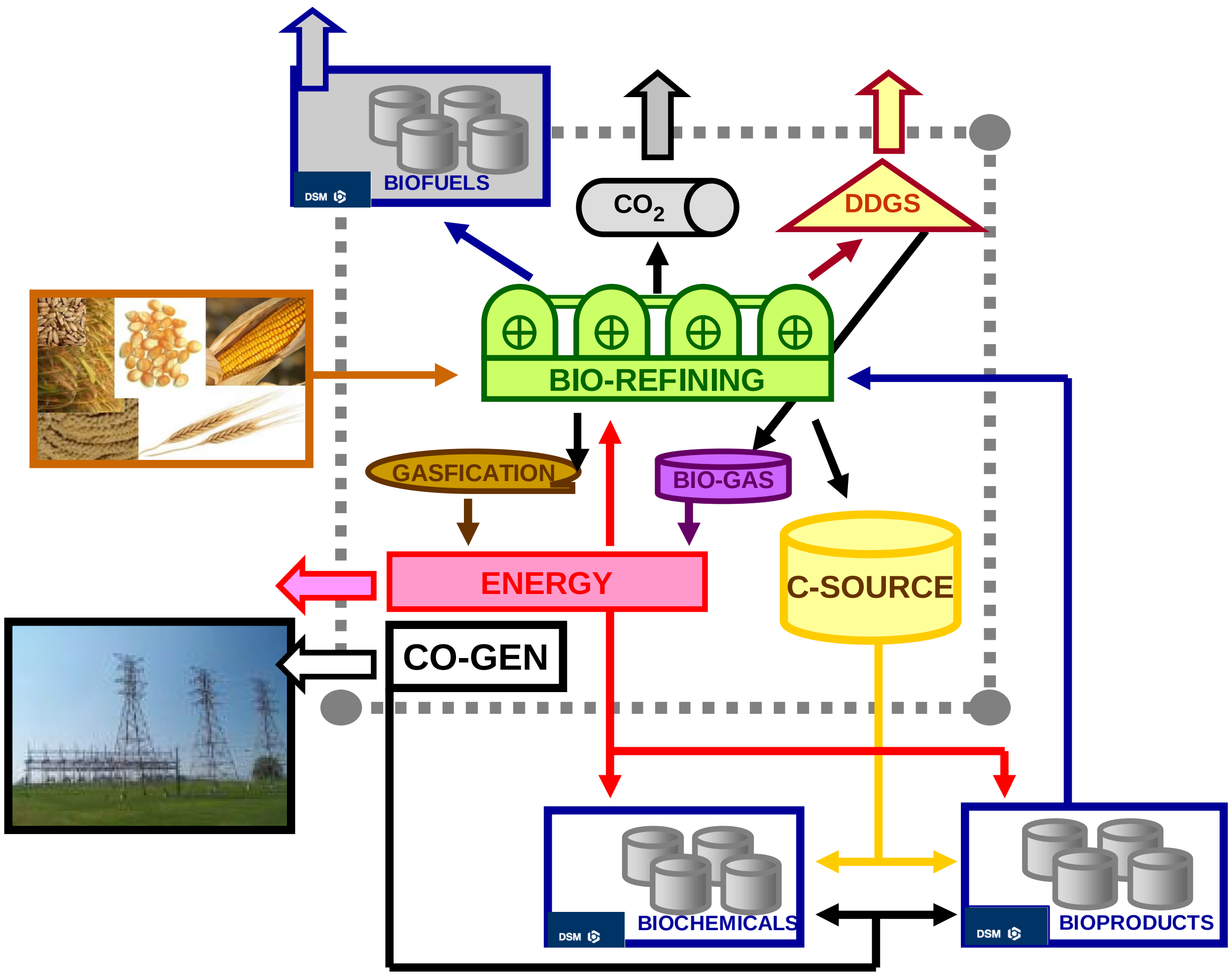
Biorefinery for Bio-based Building Blocks (Sugars)



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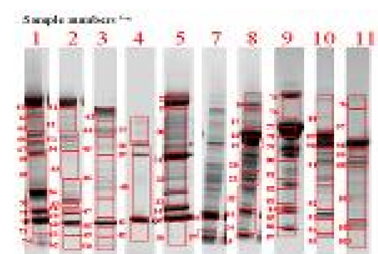
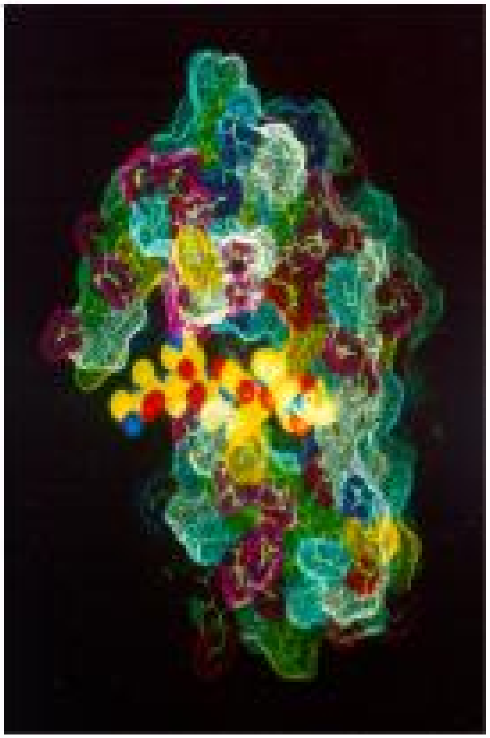
DSM has a long history in Yeast & Enzymes

- ***Classical* Biotechnology (>1870's)**
  - Yeast, Ethanol, Yeast extracts, Vitamins, Penicillin Butanol (ABE), Enzymes, Citric acid
- ***Modern* Biotechnology (>1980's)**
  - Recombinant enzymes (e.g. Chymosin, Phytase)
  - Metabolic engineering (e.g. Vitamin B_2, Cephalexin)
  - Biocatalysis (e.g. pharmaceutical intermediates)
  - Cell culture (e.g. Per·C6^{TM} human cell line)
- ***White* Biotechnology (>2006)**
  (Accelarated Innovation Program)
  - Enzymes & Yeast for next generation biorefineries
  - Renewable processes for chemicals, materials and biofuels.



Enzymes: *A Core Bio-based Building Block Platform for DSM*



- DSM possesses a strong knowledge base within enzyme research, application and manufacturing
- DSM holds hundreds of patents based on enzyme technology including biomass deconstruction enzymes
- DSM sells $300MM worth of enzymes in baking, food & fruit processing, brewing, wine, dairy, animal feed, antibiotics, flavors, and biocatalysis
- US DoE Partnership with DSM for commercial development of enzymes for lignocellulosic feed-stocks saccharification.
- DSM has a global enzyme business

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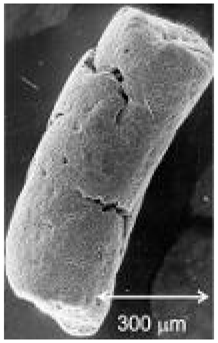
Fermentation Organisms for 2nd Generation Feedstocks:
Leveraging DSM's Baker's Yeast Technology

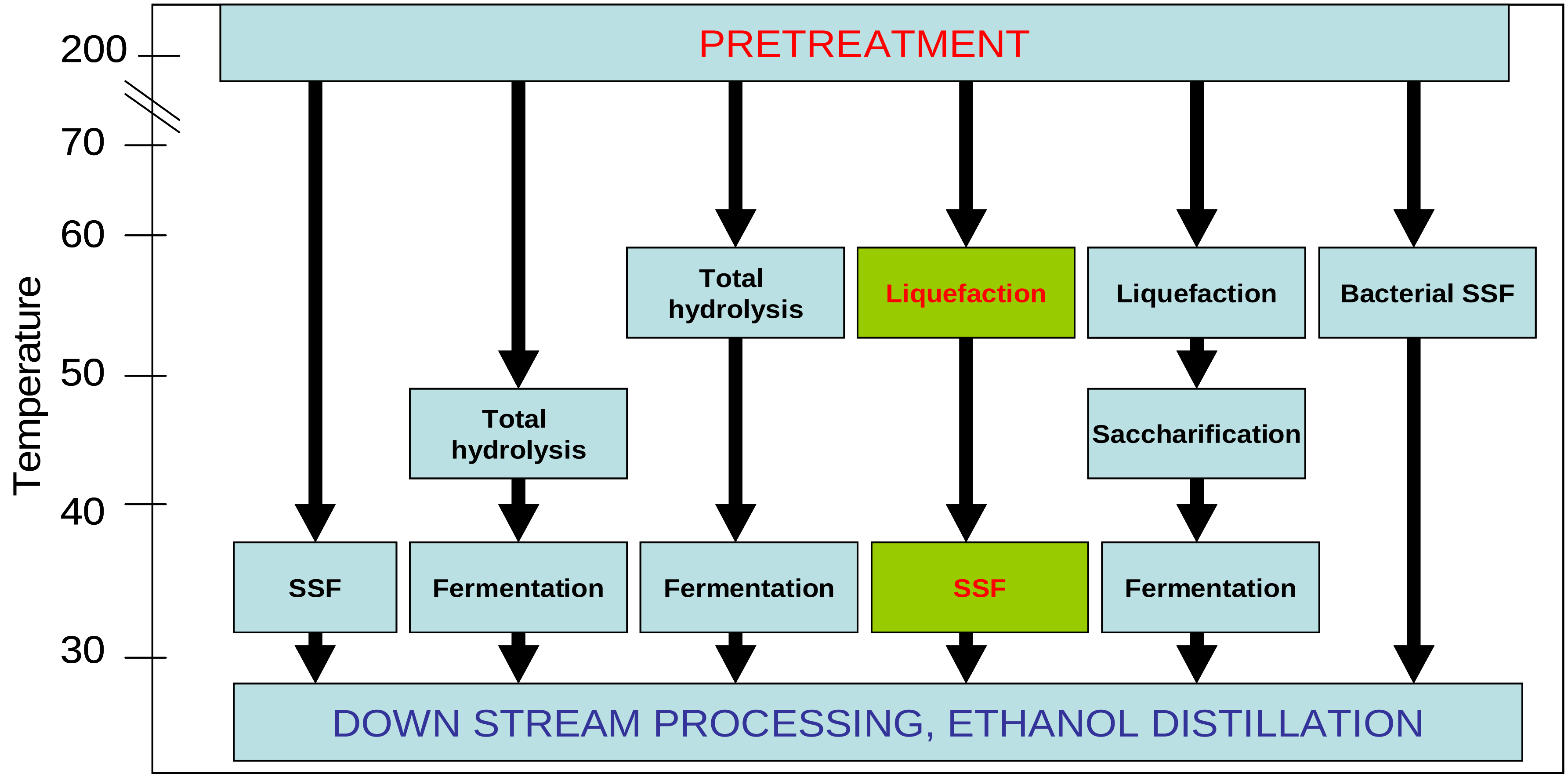
- **Traditional Ethanol Yeast**
- Long history of safe industrial use
- Fermentation and strain development tools well developed
- Robust organism
- First eukaryotic genome sequenced (1998)
- >40 MT/yr fuel ethanol applications for > 30 years
- Efficient fermentation of C6 sugars (1st generation)



- **However, *traditional* Yeast only uses C6 sugars and it does not**
- **efficiently produce BioEthanol on cellulose feedstock (C5 sugars)**

- **DSM C5 Ethanol Yeast**
- **DSM is developing Yeast that consumes cellulose hydrolysates (C5 Yeast), suited for 2nd generation biorefineries**

Configuration of various hydrolysis and fermentation processes at different temperatures using thermostable enzymes and DSM preferred choice. (SHF, SSF, HHF, HHCF, NSSF, SSCF, CBP)



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Ref: Liisa Viikari, 2007, Adv Biochem Eng. Biotech

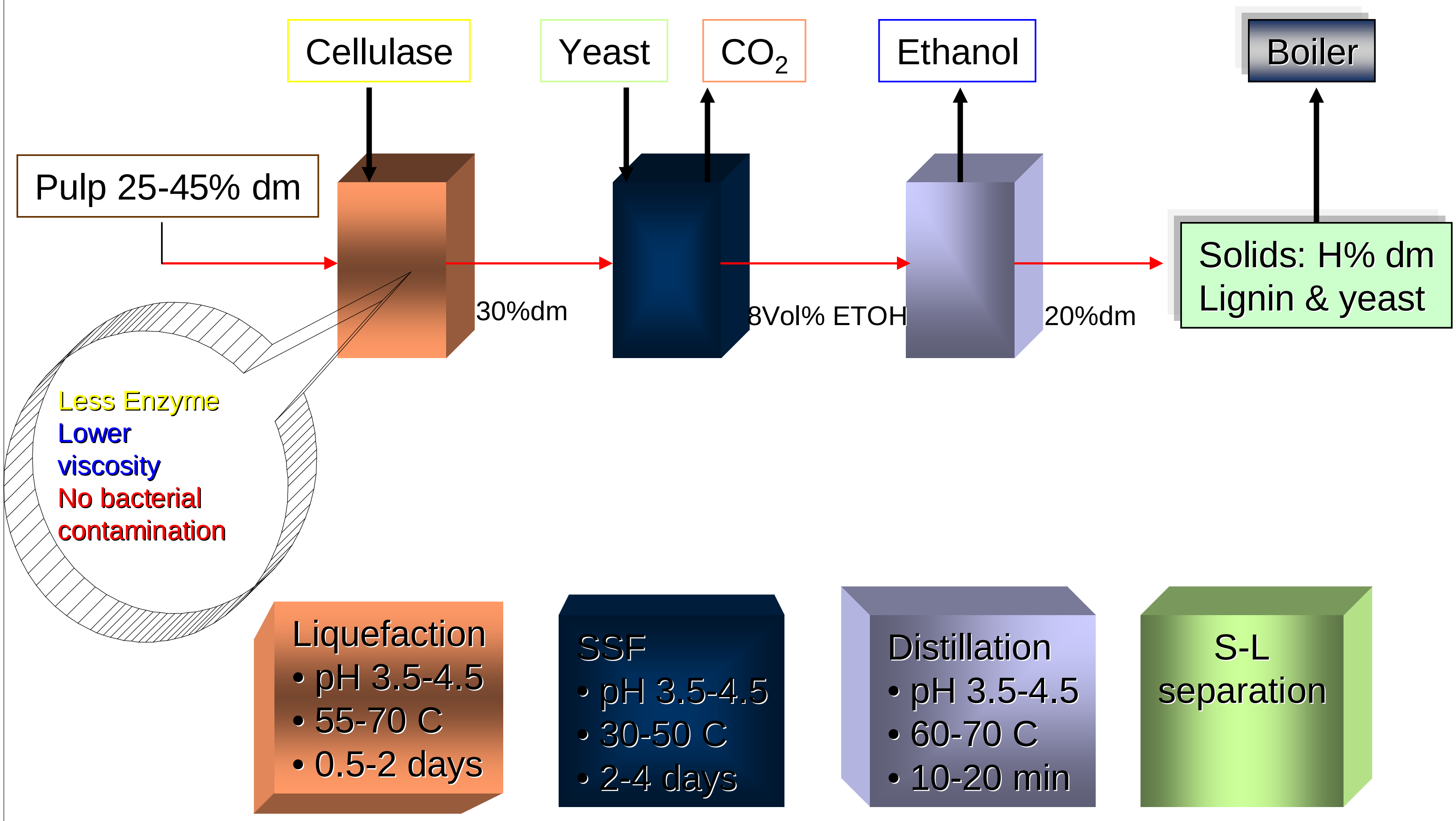
DSM Cellulosic Enzyme System Product

- Differentiated and Tailor made for Biomass saccharification
  - Peers enzymes based on *Trichoderma* technology basically originated for textile and paper industry
- Thermostable enzymes suited to work at 65C vs. 40-50C for *Trichoderma*
  - Lower dosage, no contamination, higher dry solid loading
- DSM enzyme system efficient for SSF, SHF, SHCF
- Fast viscosity reduction allowing higher DS via fed-batch
- No interference with yeast growth
- Insignificant inhibition (glucose) up to 6% w/w
- No Inhibition (ethanol) up to 8% w/w
- On-site manufacturing/ whole broth: provides enough nutrients for yeast growth

Thermostable Cellulases: *Why ?*

- Increased rate of cellulase activity, less energy cost for cooling, higher DS loading, and decreased risk of contamination.
- Can be cloned and over-expressed at high levels in *fungal hosts*.
- Ease of DSP/Formulation since one can introduce heat step to precipitate *fungal host* proteins.
- Biotransformation reactions can be carried out at higher temperatures where accessibility to substrates gets better and lignin is less tightly associated with cellulose.
- Enzymes are more robust to exposure of inhibitors and product ethanol.
- More resistant to proteolysis.

An Economically Viable Technology Package with Thermostable enzymes



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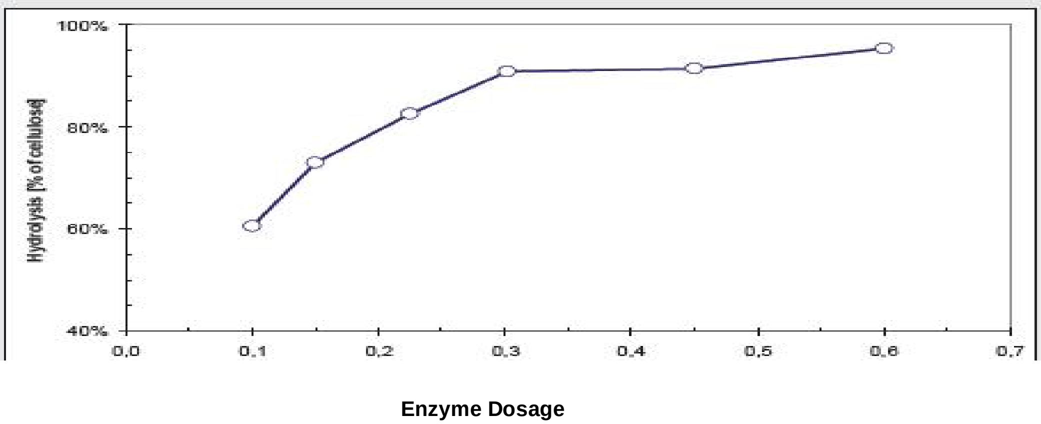
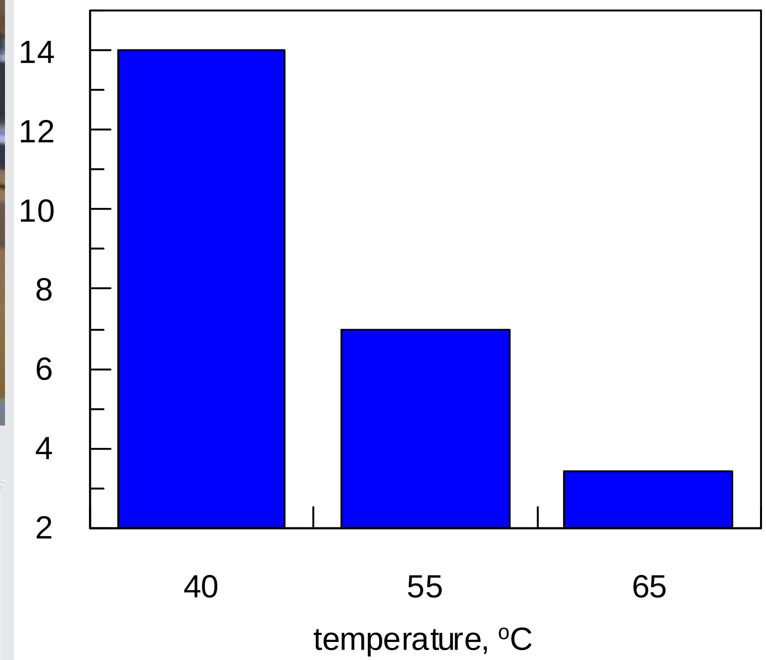
DSM Cellulosic Enzyme System Performance

Washed pWS before (left) and after (right) hydrolysis.





DSM enzymes work faster at higher temperatures

Lowering both Capital & Operating Cost

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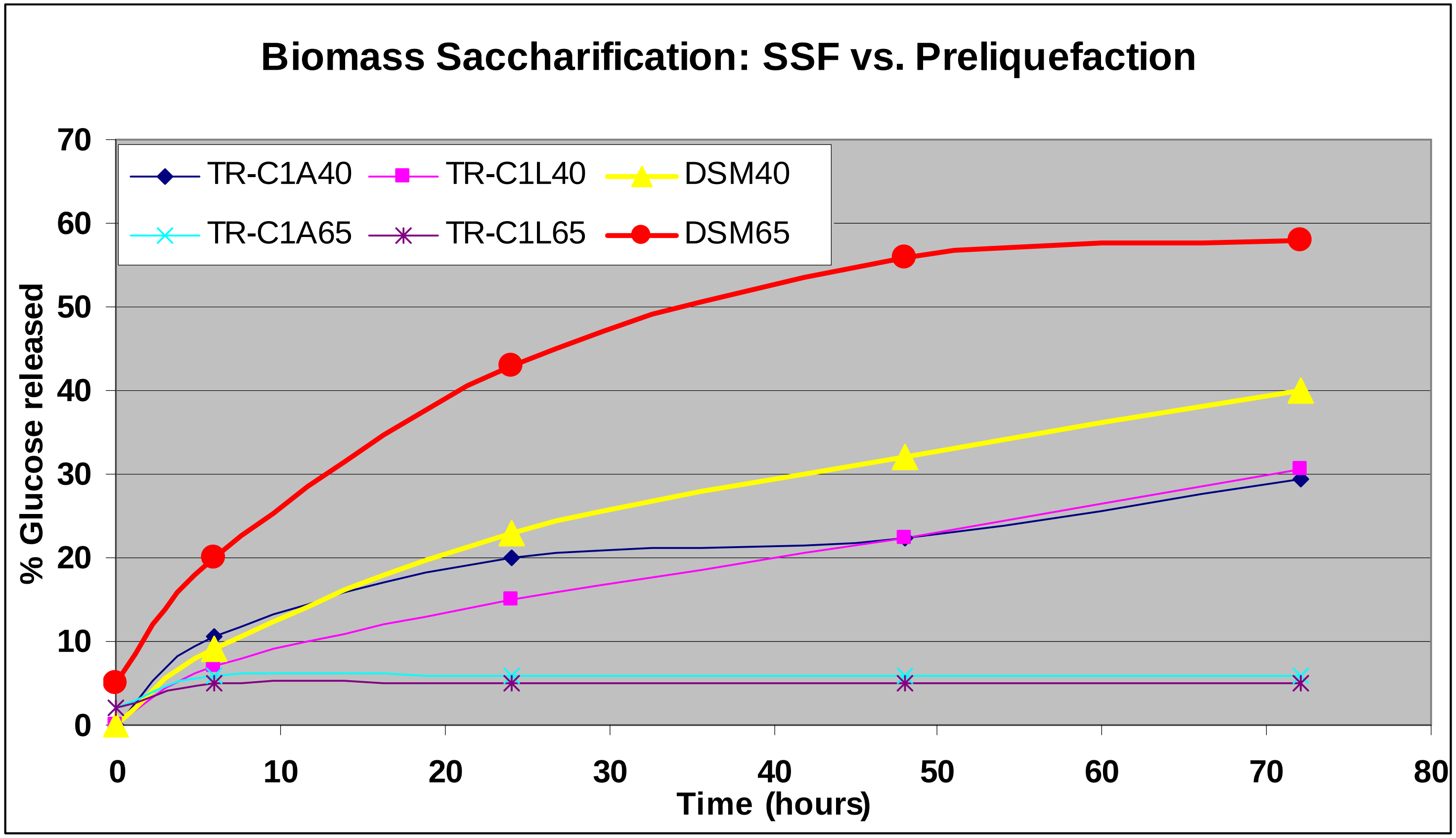
DSM Cellulase Enzyme Cocktail Performance



Successful Feed-Stock Tests with DSM Enzyme Cocktail

- Corn fiber (dilute acid; hot water; steam explosion)
- Corn stover (dilute acid)
- Wheat straw (dilute acid; hot water; steam explosion)
- Spruce (SO_2 catalyzed steam explosion)
- Switch grass (dilute acid)
- Poplar (dilute acid)

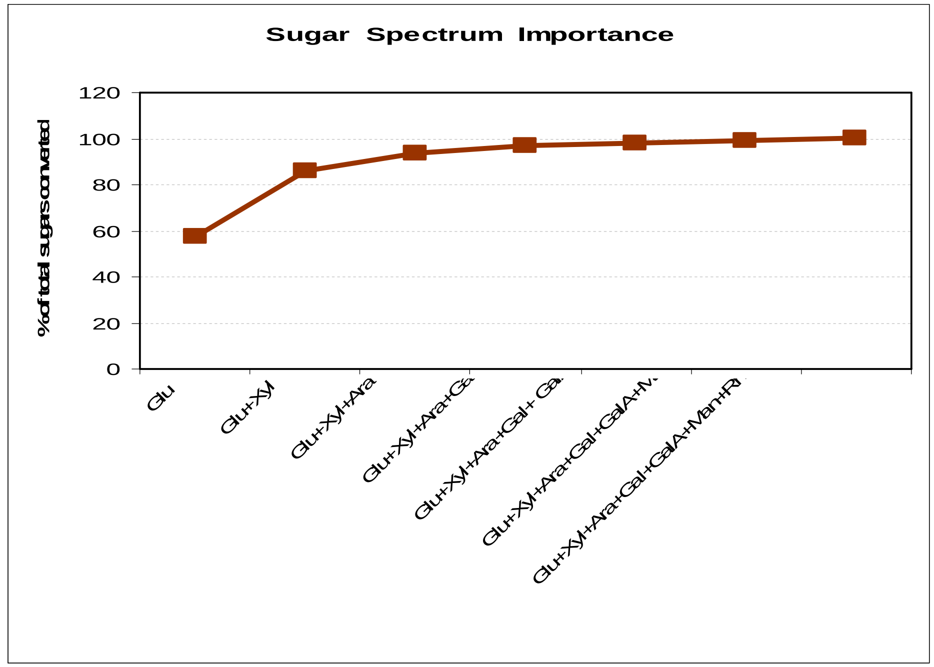
DSM Ethanologen Program: *All in One Yeast*

- DSM has now developed an industrial advanced yeast using a robust *Saccharomyces cerevisiae* host that was engineered to enable the conversion of the most abundant biomass sugars glucose, xylose, arabinose, galactose, and mannose at high yield to ethanol.
- DSM technology has emerged from research into development phase taking cellulosic ethanol a step closer to commercial realization.
- DSM Cellulosic Yeast product optimized with DSM Cellulosic enzyme cocktail to offer a full package solution to industry vs. peers.

DSM Criteria for Technology Package Selection

- Feed-stock
  - Volume, logistics, ag residues, collected, available, clean, all year around
- Pretreatment
  - Established method, hemicellulose sacchrifying, generating potential byproduct
    - H_2SO_4 (cheaper), Ammonium Hydroxide (as neutralization aid)
    - Ammonium Sulfate as fertilizer
  - Dilute acid pretreated
    - Corn Fiber
    - Corn Stover
- Hydrolysis & Fermentation
  - Thermostable enzymes, higher dry matter, low contamination
  - All in one Yeast biomass production & dosage

Five Biomass Sugar Converting Yeast



Ethanol Potential	0.511	Kg/kg sugar					
Hydrolysis Yield	90	%					
Fermentation yield	90	%					
	kg EtOH/kg galactan	kg EtOH/kg Xylan	kg EtOH/kg arabinan	kg EtOH/kg mannan	kg EtOH/kg glucan	kg EtOH/kg dm	gallons/ton dm
corn cob	24	130	42	0	115	311	104
corn fiber	17	83	53	4	171	328	110
soy hulls	41	52	5	37	202	337	113
bagasse	6	83	11	2	180	282	94
average	*19*	*99*	*29*	*9*	*169*	*324*	*108*

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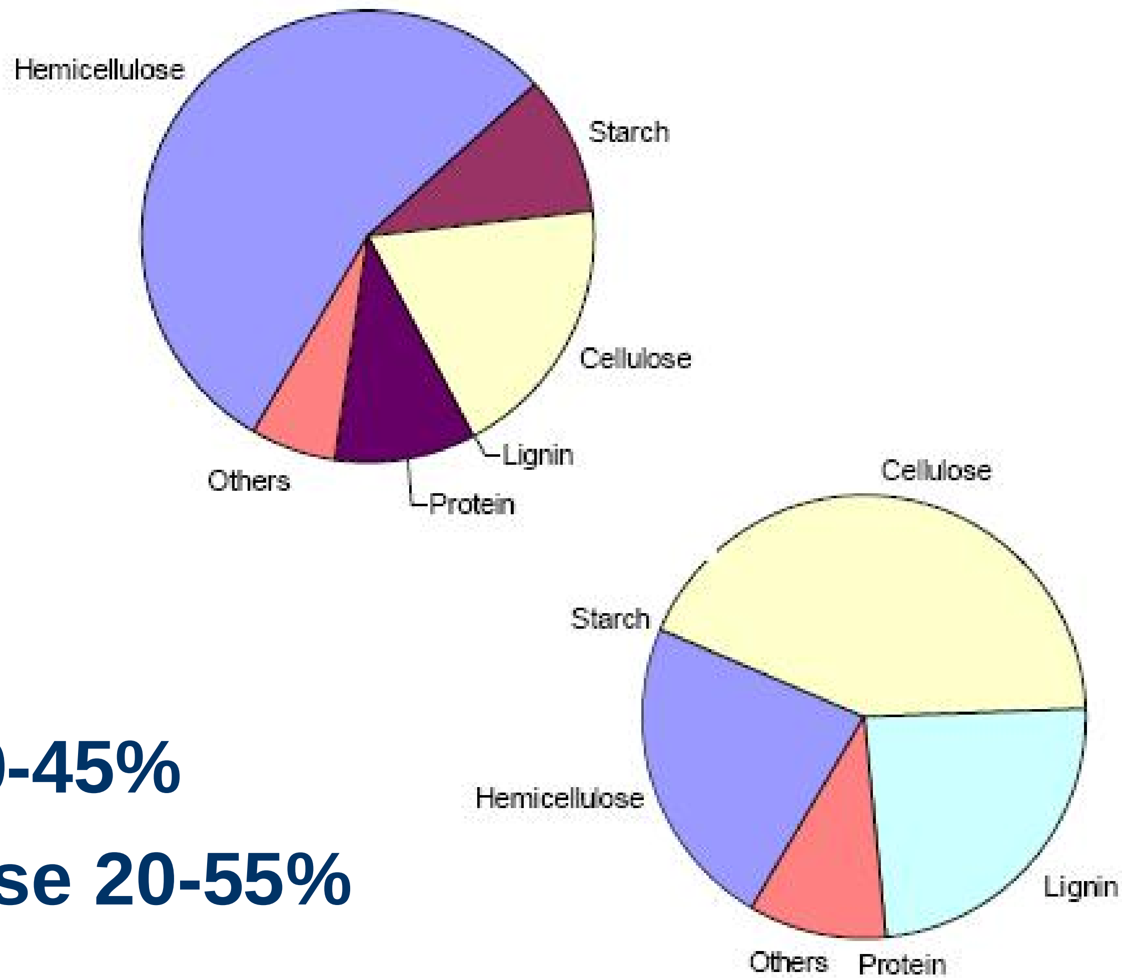
Lignocellulosic Feedstock Composition:
Corn as an Example with two diverse composition case

- Corn fiber
- Corn stover



Cellulose 20-45%

Hemicellulose 20-55%

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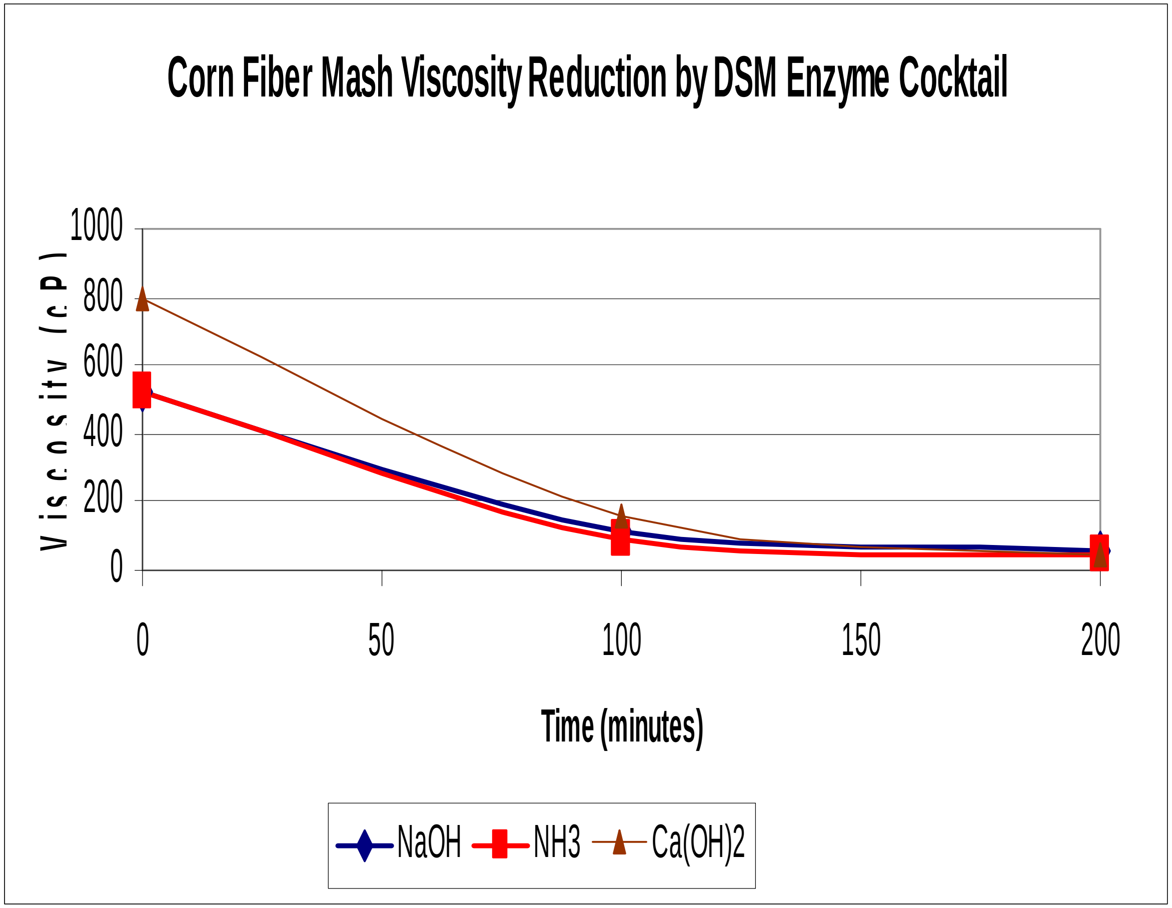
Example 1: *Application Parameters*

- Corn Fiber
  - Hydrolysis
    - 13.8 % dry matter dilute acid pretreated corn fiber
    - pH to 5.0
    - Addition of DSM Biomass Saccharification Thermostable Enzyme Cocktail
    - Liquifaction for 72 hours at 60 °C
    - Cool down to 33 °C
  - Fermentation
    - Addition of salts and anti foam
    - pH to 5.5
    - Addition of DSM Yeast
    - Fermentation at 33 °C till no CO_2 production observed anymore

13.8% DM dilute acid pretreated Corn Fiber Mash Viscosity Reduction at the Early Phase of Hydrolysis (pH 5)



- preferred base NH_3
- fertilizer ammonium sulfate
- No negative impact on viscosity reduction and sugar formation using NH_3 as base.

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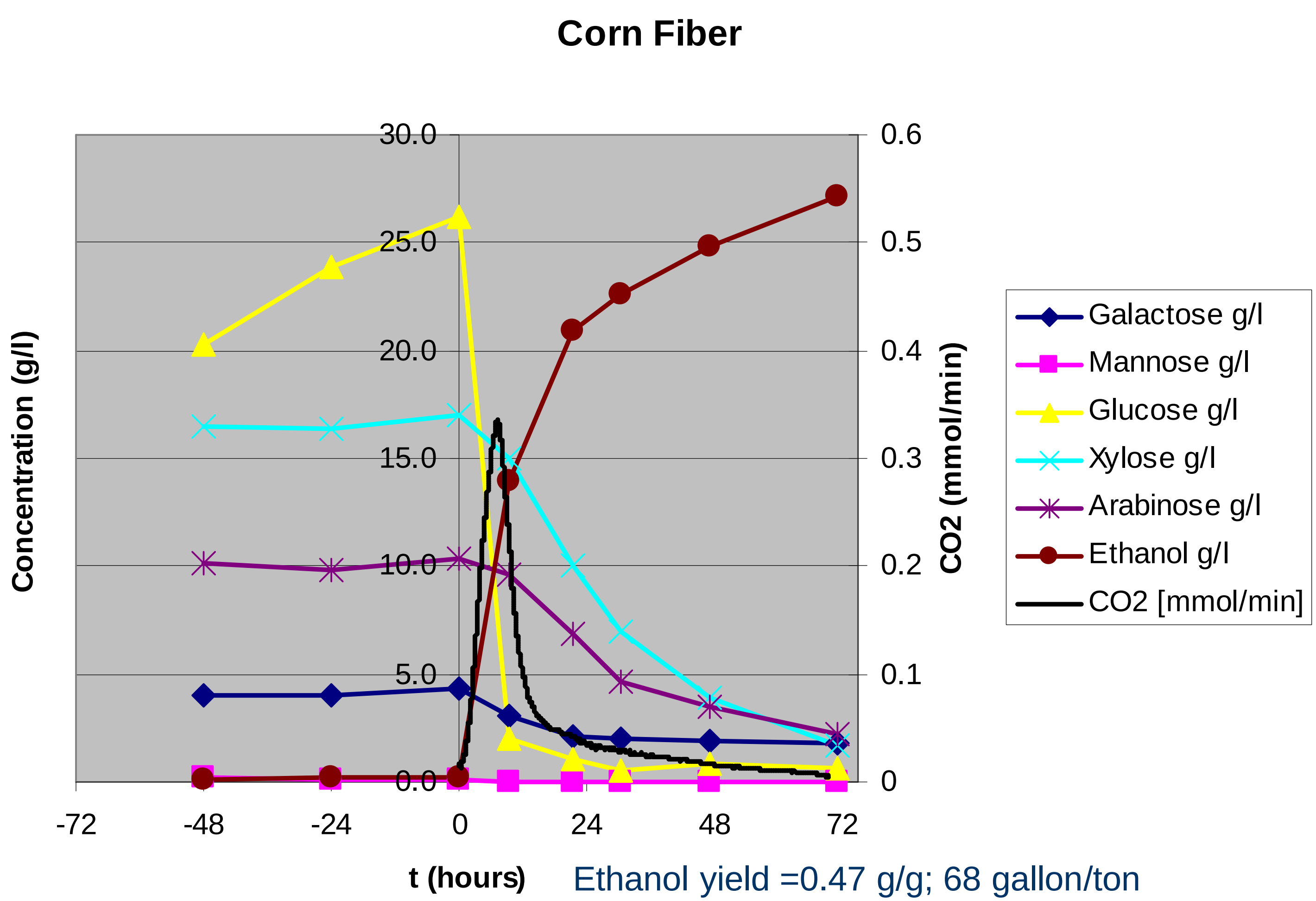
13.8% DS Corn-fiber (low severity dilute acid PT)

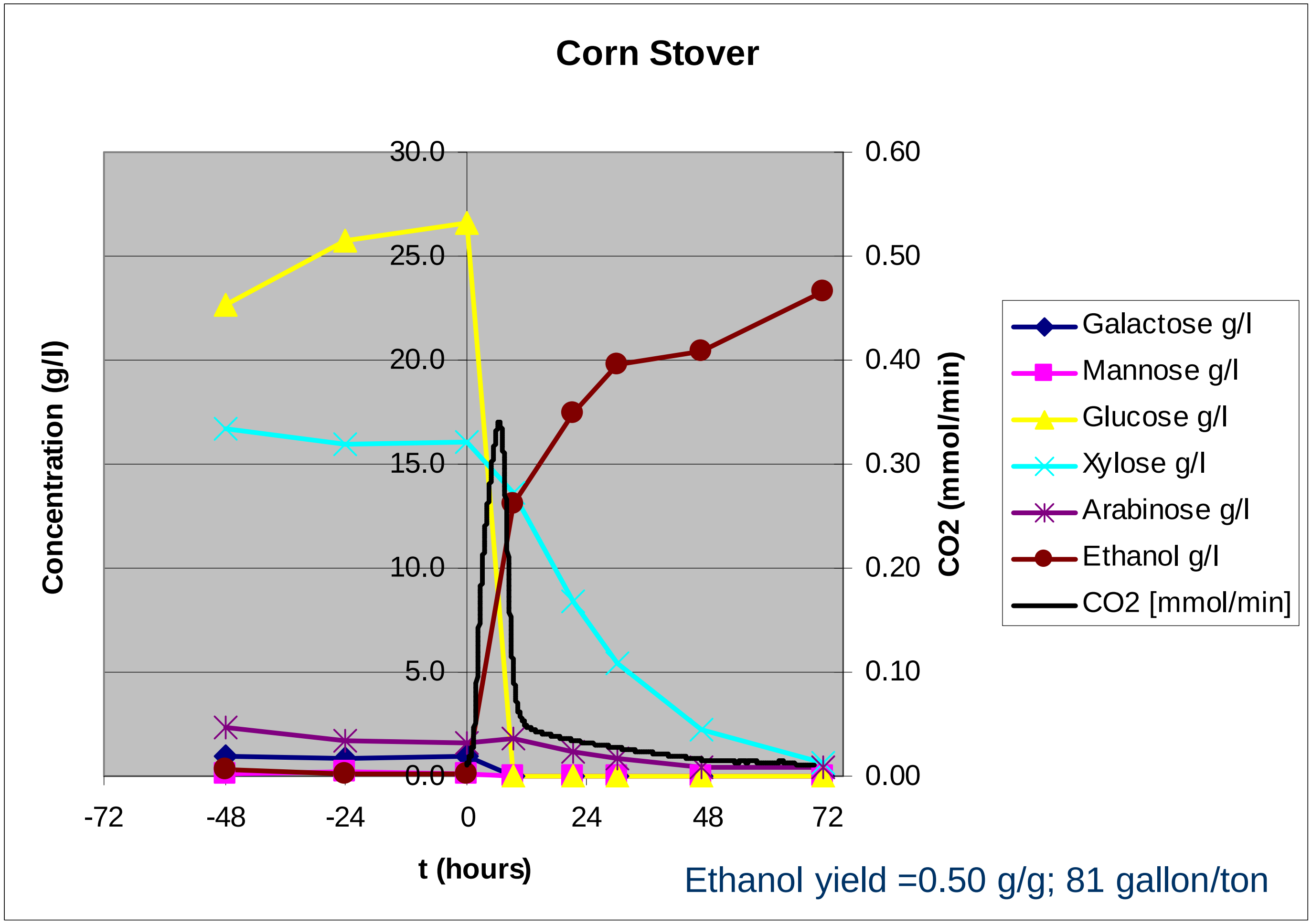


Ethanol yield =0.47 g/g; 68 gallon/ton

Example 2: *Application Parameters*

- Corn Stover
  - Hydrolysis
    - 10 % dry matter dilute acid pretreated corn stover
    - pH to 5.0
    - Addition of DSM Biomass Saccharification Thermostable Enzyme Cocktail
    - Liquifaction for 72 hours at 60 °C
    - Let it cool to 33 °C
  - Fermentation
    - Addition of salts and anti foam
    - pH to 5.5
    - Addition of DSM Yeast
    - Fermentation at 33 °C till no CO_2 production observed anymore

10% DS Corn Stover: *Enzyme & Yeast Performance*



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DSM Enzyme & Yeast On-site Production: *a lower cost and more sustainable approach*

- Traditional Production
  - Large scale and capital
  - Need for lower cost geographies
  - DSP and formulation
  - Logistics
  - Life cycle and GHG

- On-site Manufacturing
  - Smaller footprint and lower capital
  - Production on location with option of near-site supply
  - Minimal to no formulation
  - Minimal logistics issues
  - More cost and adaptable technology
  - Better LCA and lower GHG

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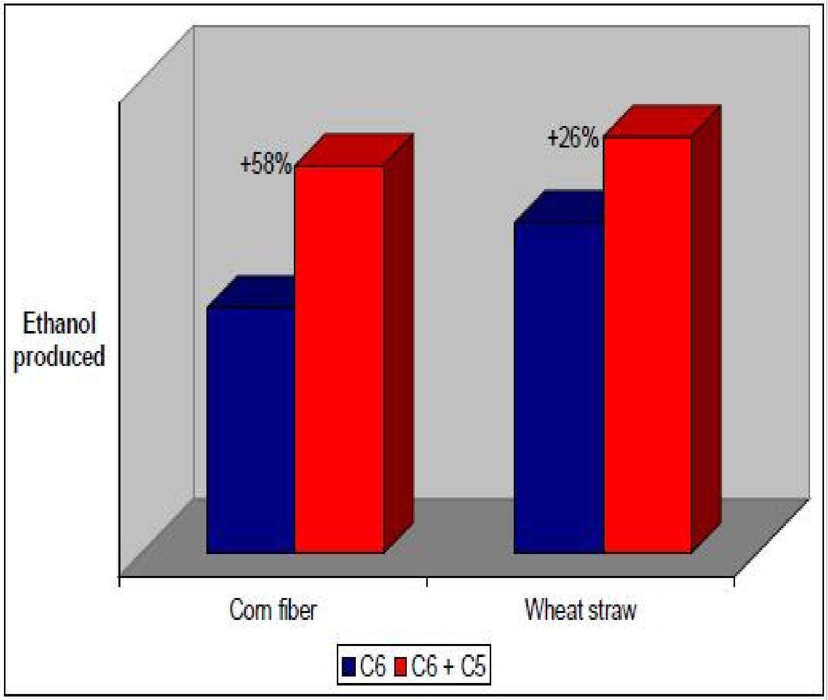
Take Home Message I: *DSM Enzyme Technology*

- **Thermo-stability** and **lower pH optimum** properties of DSM enzyme cocktail provides multiple economical, technical, and functional advantages
- DSM cellulase enzyme is ready to be used for various pretreatments and feed-stocks- production process scaled and successfully piloted
- Full saccharification >90% glucan hydrolysis in both SHF and SSF (lower enzyme load) batch process possible
- Fed batch application (feeding fibers to enzyme) at higher dry matter possible
- On site manufacturing of yeast and enzymes advantageous from an economic and LCA point of view.

Take Home Message II: *DSM All in One Yeast*

- Genes chromosomally integrated for the xylose and arabinose pathway for xylose and arabinose utilization
- Adaptation improves to meet the clean sugar milestones and shows progress for concomitant use of all C5 and C6 sugars in real biomass hydrolysates including un-dissociated acetic acid.
- Automated, continuous culture adaptation in place
  - Specific sugar adaptation
  - Growth in hydrolysate
  - Growth at low complex nutrient input
- Facile scale up process in place for marker-free yeast production from lab to commercial scale



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Acknowledgements

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